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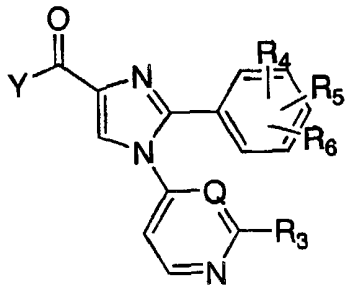

(I)

(57) Abstract

There are disclosed compounds of formula (I) and pharmaceutically acceptable salts thereof which exhibit utility for the treatment of cytokine mediated diseases such as arthritis.

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TITLE OF THE INVENTION

COMPOUNDS HAVING CYTOKINE INHIBITORY ACTIVITY

BACKGROUND OF THE INVENTION

The present invention relates to substituted imidazole compounds which have cytokine inhibitory activity. Cytokine mediated diseases and cytokine inhibition, suppression and antagonism are used in the context of diseases or conditions in which excessive or unregulated production or activity of one or more cytokines occurs. Examples of cytokines which are effected typically include Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8) and Tumor Necrosis Factor (TNF).

Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF) are produced by a variety of cells which are involved in immunoregulation and other physiological conditions.

There are many disease states in which IL-1 is implicated. Examples are rheumatoid arthritis, osteoarthritis, endotoxemia, toxic shock syndrome, acute and chronic inflammatory diseases, such as the inflammatory reaction induced by endotoxin or inflammatory bowel disease; tuberculosis, atherosclerosis, muscle degeneration, cachexia, psoriatic arthritis, Reiter's syndrome, rheumatoid arthritis, gout, traumatic arthritis, rubella arthritis and acute synovitis. Recent evidence also links IL-1 activity to diabetes.

Interleukin-1 has been demonstrated to mediate a variety of biological activities thought to be important in immunoregulation and other physiological conditions. [See, e.g., Dinarello et al., Rev. Infect. Disease, 6, 51 (1984)]. The known biological activities of IL-1 include the activation of T helper cells, induction of fever, stimulation of prostaglandin or collagenase production, neutrophil chemotaxis, induction of acute phase proteins and the suppression of plasma iron levels.

Excessive or unregulated tumor necrosis factor (TNF) production or activity has been implicated in mediating or exacerbating rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis, and other arthritic conditions, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome,

adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcosis, bone resorption diseases, reperfusion injury, graft v. host rejection, allograft rejections, fever and myalgia due to infection, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), AIDS related complex (ARC), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis and pyresis.

Monokines, such as TNF, have also been shown to activate HIV replication in monocytes and/or macrophages [See Poli, *et al.*, Proc. Natl. Acad. Sci., 87:782-784 (1990)], therefore, inhibition of monokine production or activity aids in limiting HIV progression. TNF has been implicated in various roles with other viral infections, such as the cytomegalovirus (CMV), influenza virus and the herpes virus.

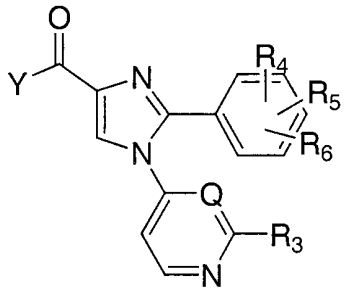
Interleukin-6 (IL-6) is a cytokine effecting the immune system and hematopoiesis. It is produced by several mammalian cell types in response to agents such as IL-1, and is correlated with disease states such as angiofollicular lymphoid hyperplasia.

Interleukin-8 (IL-8) is a chemotactic factor first identified and characterized in 1987. Many different names have been applied to IL-8, such as neutrophil attractant/activation protein-1 (NAP-1), monocyte derived neutrophil chemotactic factor (MDNCF), neutrophil activating factor (NAF), and T-cell lymphocyte chemotactic factor. Like IL-1, IL-8 is produced by several cell types, including mononuclear cells, fibroblasts, endothelial cells and ketainocytes. Its production is induced by IL-1, TNF and by lipopolysaccharide (LPS). IL-8 stimulates a number of cellular functions *in vitro*. It is a chemoattractant for neutrophils, T-lymphocytes and basophils. It induces histamine release from basophils. It causes lysozomal enzyme release and respiratory burst from neutrophils, and it has been shown to increase the surface expression of Mac-1 (CD11b/CD 18) on neutrophils without *de novo* protein synthesis.

There remains a need for compounds which are useful in treating cytokine mediated diseases, and as such, inhibit, suppress or antagonize the production or activity of cytokines such as IL-1, IL-6, IL-8 and TNF.

SUMMARY OF THE INVENTION

The invention relates to compound I of the formula



(I)

wherein

Y is OH, O(C_1- C_6 alkyl), NR_1R_2, N(C_1- C_6 alkyl), each alkyl being optionally substituted by 1-3 groups selected from halogen, hydroxy, CF_3, NH_2, and NO_2; or a heterocyclic group connected to the carbonyl group by a direct bond, NH or by C_1-C_6 alkyl, with the proviso that both R_1 and R_2 cannot be hydrogen;

R_1 and R_2 are independently hydrogen, C_1-C_6 alkyl, (C_1- C_6 alkyl)-N-(C_1- C_6 alkyl)$_p$; said alkyl being optionally substituted by 1-3 groups selected from halogen, hydroxy, CF_3, NH_2, and NO_2; or

R_1 and R_2 are taken together to form an optionally substituted 4 to 10 membered mono or bicyclic heterocycle ring containing at least one N atom, and optionally containing 1-2 additional N atoms and 0-1 O or S atoms, said ring optionally substituted by 1-3 groups selected from C_1- C_4alkyl, OH, O(C_1- C_6 alkyl);

R_3 is hydrogen, NH(C_1-C_6 alkyl)aryl, NH(C_1-C_6 alkyl) or NH(C_3-C_6 cycloalkyl), said aryl group being optionally substituted by 1-3 groups selected from halogen, hydroxy, CF_3, NH_2, and NO_2;

R_4, R_5 and R_6 independently represent a member selected from the group consisting of hydrogen, halogen, hydroxy, CF_3, NH2, NO_2, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl or aryl;

Q is CH or N;

p is 0-2;

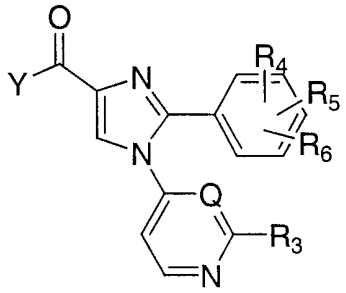
or a pharmaceutically acceptable addition salt and/or hydrate thereof, or where applicable, a geometric or optical isomer or racemic mixture thereof.

This invention also relates to a pharmaceutical composition which is comprised of a compound of formula I as defined above in combination with a pharmaceutically acceptable carrier.

Also included in the invention is a method of treating a cytokine mediated disease in a mammal, comprising administering to a mammalian patient in need of such treatment an amount of a compound of formula I which is effective for treating said cytokine mediated disease.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to compound I of the formula



(I)

wherein

Y is OH, O(C_1- C_6 alkyl), NR_1R_2, N(C_1- C_6 alkyl), each alkyl being optionally substituted by 1-3 groups selected from halogen, hydroxy, CF_3, NH_2, and NO_2; or a heterocyclic group connected to the carbonyl group by a direct bond, NH or by C_1-C_6 alkyl, with the proviso that both R_1 and R_2 cannot be hydrogen;

R_1 and R_2 are independently hydrogen, C_1-C_6 alkyl, (C_1-C_6 alkyl)-N-(C_1-C_6 alkyl)$_p$; said alkyl being optionally substituted by 1-3 groups selected from halogen, hydroxy, CF_3, NH_2, and NO_2; or

R_1 and R_2 are taken together to form an optionally substituted 4 to 10 membered mono or bicyclic heterocycle ring containing at least one N atom, and optionally containing 1-2 additional N atoms and 0-1 O or S atoms, said ring optionally substituted by 1-3 groups selected from C_1- C_4alkyl, OH, O(C_1- C_6 alkyl),

R_3 is hydrogen, NH(C_1-C_6 alkyl)aryl, NH(C_1-C_6 alkyl) or NH(C_3-C_6 cycloalkyl), said aryl group being optionally substituted by 1-3 groups selected from halogen, hydroxy, CF_3, NH_2, and NO_2;

R_4, R_5 and R_6 independently represent a member selected from the group consisting of hydrogen, halogen, hydroxy, CF_3, NH_2, NO_2, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl or aryl;

Q is CH or N;

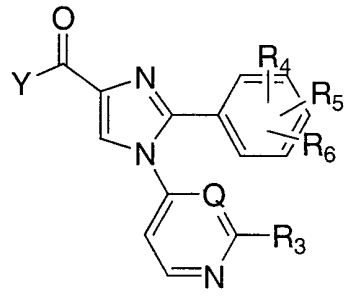
p is 0-2;

or a pharmaceutically acceptable addition salt and/or hydrate thereof, or where applicable, a geometric or optical isomer or racemic mixture thereof.

This invention also relates to a pharmaceutical composition which is comprised of a compound of formula I as defined above in combination with a pharmaceutically acceptable carrier.

Also included in the invention is a method of treating a cytokine mediated disease in a mammal, comprising administering to a mammalian patient in need of such treatment an amount of a compound of formula I which is effective for treating said cytokine mediated disease.

In a preferred embodiment, there is disclosed a compound of the formula



(I)

or a pharmaceutically acceptable salt, or optical isomer thereof,

wherein:

Y is OH, O(C_1-C_6 alkyl), NR_1R_2 or a piperidinyl or piperazinyl group connected to the imidazole ring by a direct bond;

R_1 and R_2 are independently hydrogen or $(CH_3)_2$ - N- $(CH_3)_2$; or

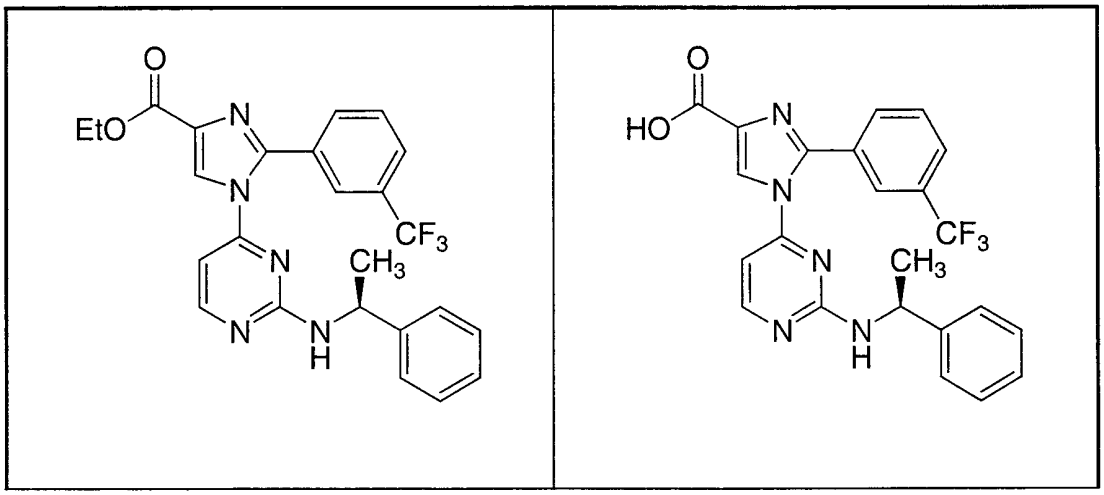
R_1 and R_2 are taken together to form a heterobicylic ring;

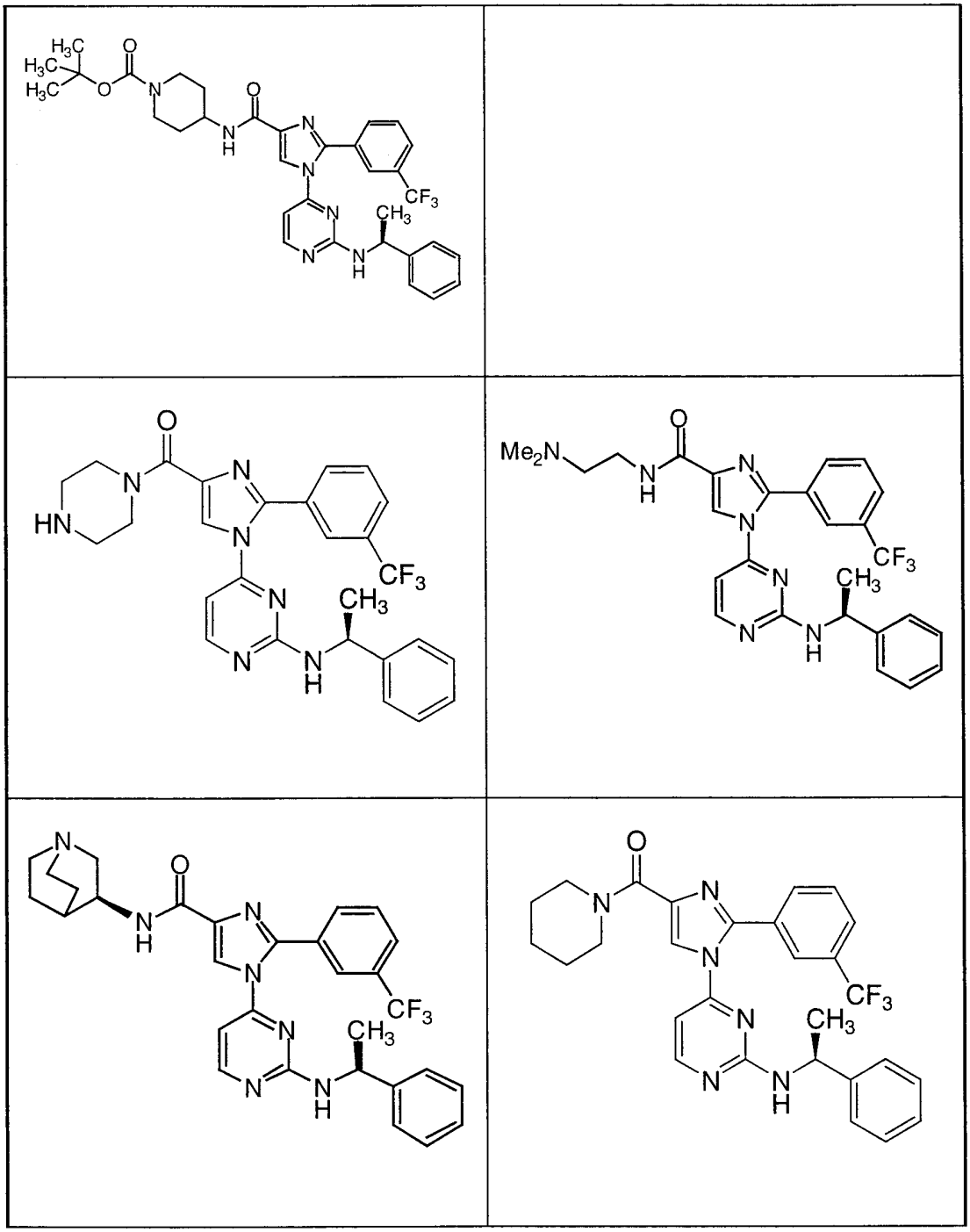
R_4 is CF_3

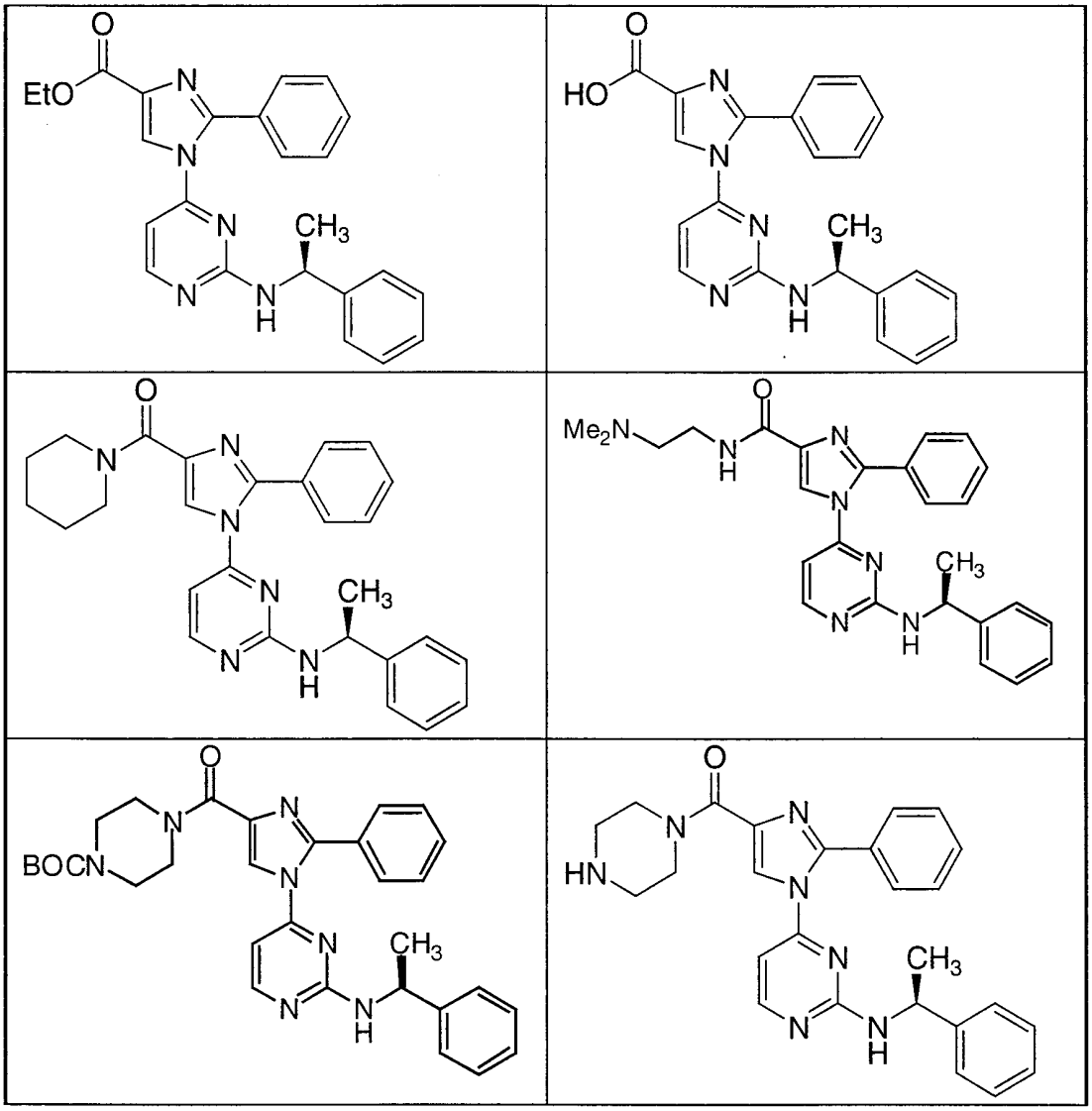
R_5 and R_6 are hydrogen; and

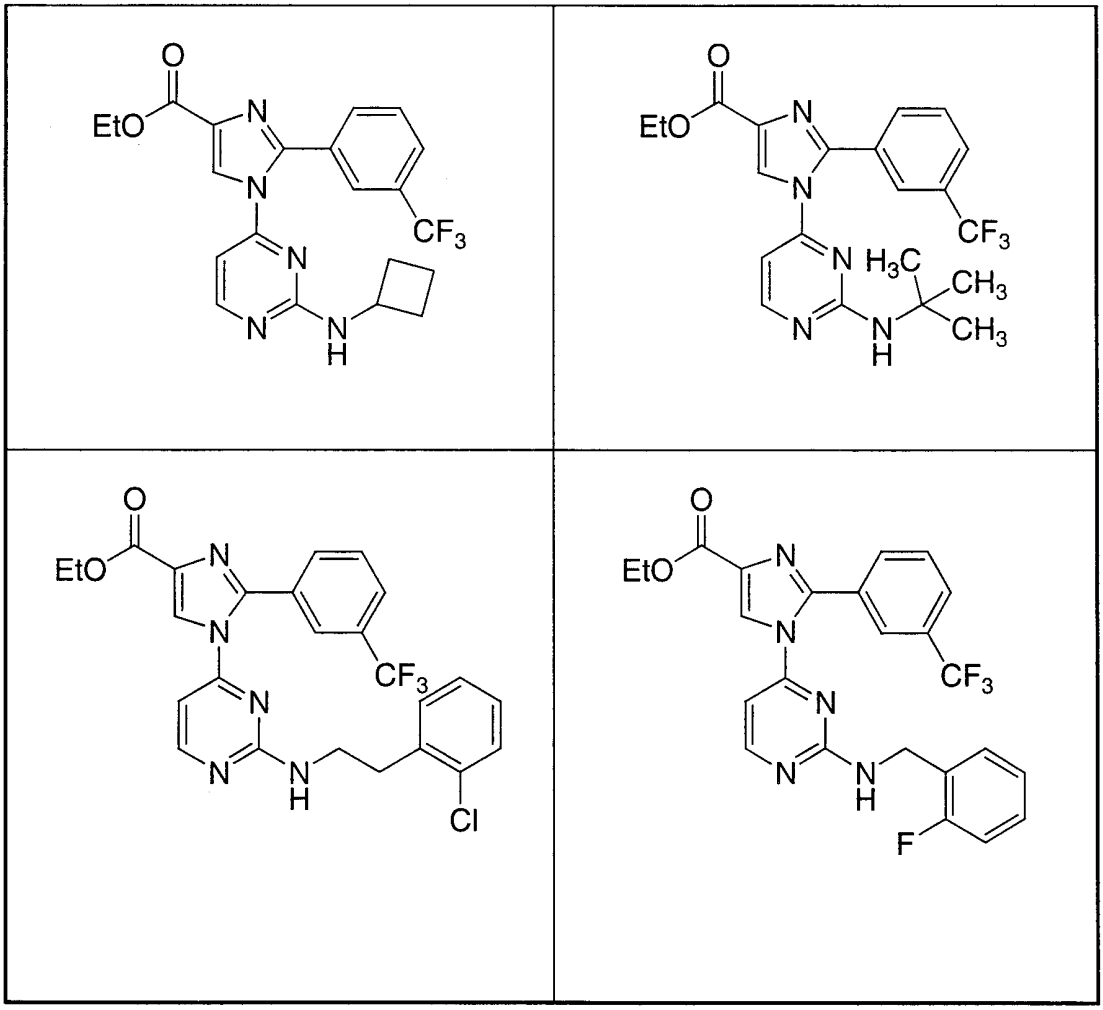
Q is N.

Representative species falling within the present invention include the following:









Unless otherwise stated or indicated, the following definitions shall apply throughout the specification and claims.

The term "alkyl" refers to a monovalent alkane (hydrocarbon) derived radical containing from 1 to 15 carbon atoms unless otherwise defined. It may be straight or branched, and when of sufficient size, e.g., C_{3-15}, may be cyclic. Preferred straight or branched alkyl groups include methyl, ethyl, propyl, isopropyl, butyl and t-butyl. Preferred cycloalkyl groups include cyclopropyl, cyclopentyl and cyclohexyl.

Alkyl also includes an alkyl group substituted with a cycloalkyl group, such as cyclopropylmethyl.

The alkylene and monovalent alkyl portion(s) of the alkyl group can be attached at any available point of attachment to the cycloalkylene portion.

When substituted alkyl is present, this refers to a straight, branched or cyclic alkyl group as defined above, substituted with 1-3 groups as defined with respect to each variable.

The term "aryl" refers to aromatic rings e.g., phenyl, substituted phenyl and like groups as well as rings which are fused, e.g., naphthyl and the like. Aryl thus contains at least one ring having at least 6 atoms, with up to two such rings being present, containing up to 10 atoms therein, with alternating (resonating) double bonds between adjacent carbon atoms. The preferred aryl groups are phenyl and naphthyl. Aryl groups may likewise be substituted as defined below. Preferred substituted aryls include phenyl or naphthyl substituted with one or two groups.

The terms "heterocycloalkyl" and "heterocyclyl" refer to a cycloalkyl group (nonaromatic) in which one of the carbon atoms in the ring is replaced by a heteroatom selected from O, S(O)y or N, and in which up to three additional carbon atoms may be replaced by said heteroatoms. When three heteroatoms are present in the heterocycle, they are not all linked together.

Examples of heterocyclyls are piperidinyl, morpholinyl, azetidinyl, pyrrolidinyl, tetrahydrofuranyl, imidazolinyl, piperazinyl, pyrolidin-2-one, piperidin-2-one and the like.

The term "halogen" or "halo" is intended to include fluorine, chlorine, bromine and iodine.

As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts.

In addition, it is well known to those skilled in the art that many of the foregoing heterocyclic groups can exist in more than one tautomeric form. It is intended that all such tautomers be included within the ambit of this invention.

The optical isomeric forms, that is mixtures of enantiomers, e.g., racemates, or diastereomers as well as individual enantiomers or diastereomers of the instant compound are included. These individual enantiomers are commonly designated according to the optical rotation they effect by the symbols (+) and (-), (L) and (D), (l) and (d) or combinations thereof. These isomers may also be designated according to their absolute spatial configuration by (S) and (R), which stands for sinister and rectus, respectively.

The individual optical isomers may be prepared using conventional resolution procedures, e.g., treatment with an appropriate optically active acid, separating the diastereomers and then recovering the desired isomer. In addition, the individual optical isomers may be prepared by asymmetric synthesis.

Additionally, a given chemical formula or name shall encompass pharmaceutically acceptable addition salts thereof and solvates thereof, such as hydrates.

The compounds of the present invention, while effective themselves, may be formulated and administered in the form of their pharmaceutically acceptable addition salts for purposes of stability, convenience of crystallization, increased solubility and other desirable properties.

The compounds of the present invention may be administered in the form of pharmaceutically acceptable salts. The term "pharmaceutically acceptable salt" is intended to include all acceptable salts. Examples of acid salts are hydrochloric, nitric, sulfuric, phosphoric, formic, acetic, trifluoroacetic, propionic, maleic, succinic, malonic, methane sulfonic and the like which can be used as a dosage form for modifying the solubility or hydrolysis characteristics or can be used in sustained release or prodrug formulations. Depending on the particular functionality of the compound of the present invention, pharmaceutically acceptable salts of the compounds of this invention include those formed from cations such as sodium, potassium, aluminum, calcium, lithium, magnesium, zinc, and from bases such as ammonia, ethylenediamine, N-methyl-glutamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylenediamine, chloroprocaine, diethanolamine, procaine, N-benzylphenethylamine, diethylamine, piperazine, tris(hydroxymethyl)aminomethane, and tetramethyl-

ammonium hydroxide. These salts may be prepared by standard procedures, e.g. by reacting a free acid with a suitable organic or inorganic base, or alternatively by reacting a free base with a suitable organic or inorganic acid.

Also, in the case of an acid (-COOH) or alcohol group being present, pharmaceutically acceptable esters can be employed, e.g. methyl, ethyl, butyl, acetate, maleate, pivaloyloxymethyl, and the like, and those esters known in the art for modifying solubility or hydrolysis characteristics for use as sustained release or prodrug formulations.

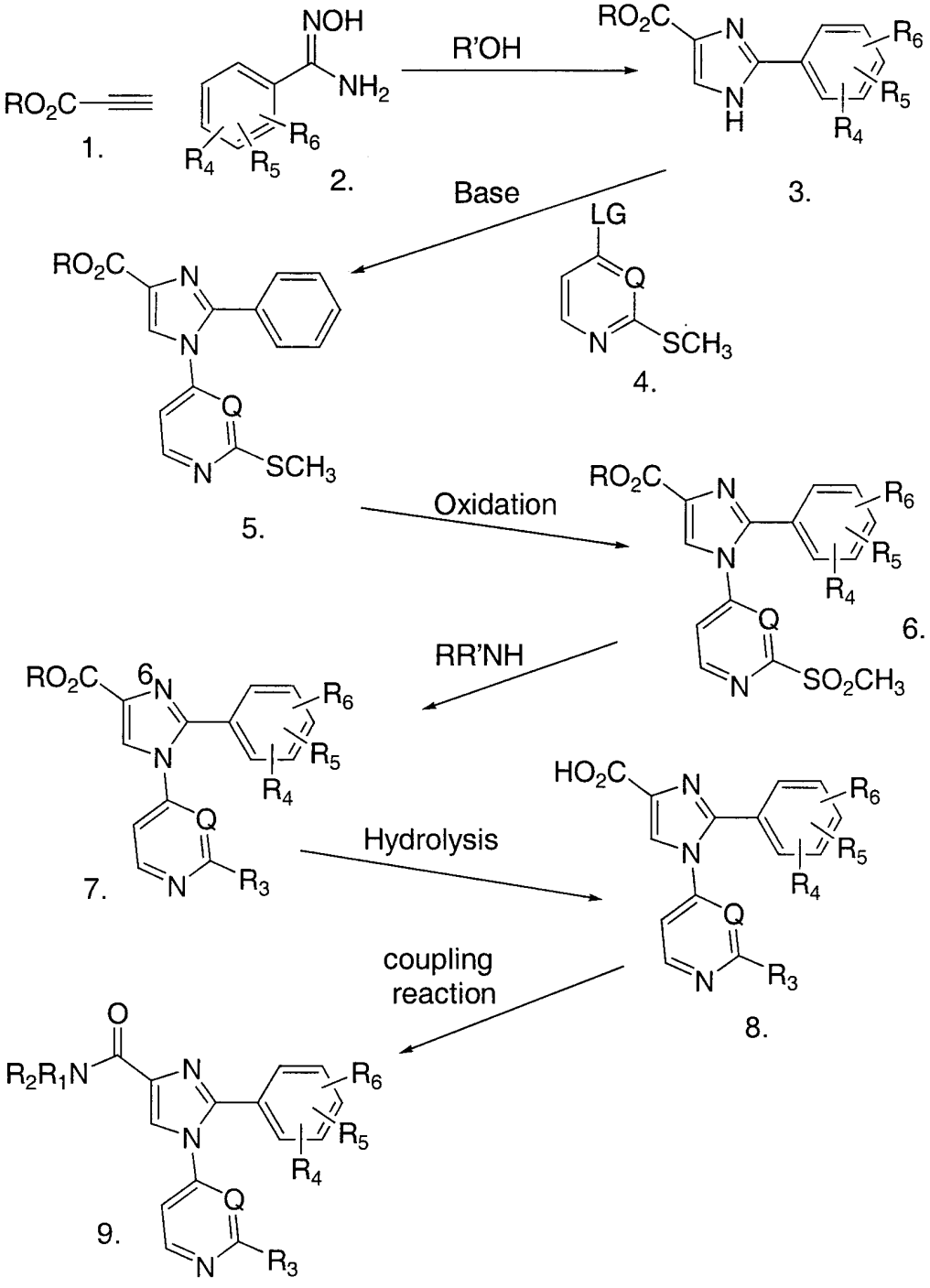
The compounds of the present invention may have chiral centers other than those centers whose stereochemistry is depicted in formula I, and therefore may occur as racemates, racemic mixtures and as individual enantiomers or diastereomers, with all such isomeric forms being included in the present invention as well as mixtures thereof. Furthermore, some of the crystalline forms for compounds of the present invention may exist as polymorphs and as such are intended to be included in the present invention. In addition, some of the compounds of the instant invention may form solvates with water or common organic solvents. Such solvates are encompassed within the scope of this invention.

The term "TNF mediated disease or disease state" refers to disease states in which TNF plays a role, either by production or increased activity levels of TNF itself, or by causing another cytokine to be released, such as but not limited to IL-1 or IL-6. A disease state in which IL-1, for instance is a major component, and whose production or action, is exacerbated or secreted in response to TNF, would therefore be considered a disease state mediated by TNF.

The term "cytokine" as used herein means any secreted polypeptide that affects the functions of cells and is a molecule which modulates interactions between cells in the immune, inflammatory or hematopoietic response. A cytokine includes, but is not limited to, monokines and lymphokines regardless of which cells produce them. Examples of cytokines include, but are not limited to, Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Tumor Necrosis Factor-alpha (TNF-α) and Tumor Necrosis Factor-beta (TNF-β).

By the term "cytokine interfering or cytokine suppresive amount" is meant an effective amount of a compound of formula I which will cause a decrease in the *in vivo* activity or level of the cytokine to normal or sub-normal levels, when given to the patient for the prophylaxis or therapeutic treatment of a disease state which is exacerbated by, or caused by, excessive or unregulated cytokine production or activity.

The compounds of the invention are prepared by the following reaction schemes. All substituents are as defined above unless indicated otherwise. R and R' are Y.


NOH
NH2
R'OH
RO2C
R6
R5
R4
N
H
1.
2.
3.
Base
LG
Q
SCH3
4.
5.
Oxidation
SO2CH3
6.
RR'NH
6
R3
7.
Hydrolysis
HO2C
8.
coupling
reaction
O
R2R1N
9.

Reaction of an ester of propiolic acid 1 with an appropriately substituted aryl amide oxime 2 by heating together in a solvent such as an alcohol provides the imidazole 3. Treatment with base and reaction with a heteroaryl compound 4 in which LG represents a suitable leaving group such as halogen affords the trisubstituted imidazole 5. The methylthio substituent of the heterocycle is then oxidized to the corresponding sulfone 6 utilizing an oxidizing agent such as Oxone or 3-chloroperoxybenzoic acid. Treatment of the sulfone 6 with an amine either neat or in the a suitable solvent, for example an alcohol or dimethylformamide yields the aminoheterocycle 7. Hydrolysis of the ester group of 7 is accomplished by treatment with base and the resulting acid 8 can then be coupled with an appropriate amine by utilizing one of the many coupling agents known to those skilled in the art, for example benzotriazol-1-yloxy-tris-(dimethylamino)-phosphonium-hexafluoro phosphate, dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide to give amides 9.

The compounds of formula 1 can be used in the prophylactic or therapeutic treatment of disease states in mammals which are exacerbated or caused by excessive or unregulated cytokines, e.g., IL-1, IL-6, IL-8 or TNF.

Because the compounds of formula I inhibit cytokines, the compounds are useful for treating diseases in which cytokine presence or activity is implicated, such as rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions.

The compounds of formula I are useful to treat disease states mediated by excessive or unregulated TNF production or activity. Such diseases include, but are not limited to sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption diseases, such as osteoporosis, reperfusion injury, graft v. host rejection, allograft rejection, fever, myalgia due to infection, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), AIDS, ARC (AIDs related complex), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis, pyresis, AIDS and other viral infections, such as

cytomegalovirus (CMV), influenza virus, and the herpes family of viruses such as Herpes Zoster or Simplex I and II.

The compounds of formula I are also useful topically in the treatment of inflammation such as in the treatment of rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions; inflamed joints, eczema, psoriasis or other inflammatory skin conditions such as sunburn; inflammatory eye conditions including conjunctivitis; pyresis, pain and other conditions associated with inflammation.

The compounds of formula I are also useful in treating diseases characterized by excessive IL-8 activity. These disease states include psoriasis, inflammatory bowel disease, asthma, cardiac and renal reperfusion injury, adult respiratory distress syndrome, thrombosis and glomerulonephritis.

The invention thus includes a method of treating psoriasis, inflammatory bowel disease, asthma, cardiac and renal reperfusion injury, adult respiratory distress syndrome, thrombosis and glomerulonephritis, in a mammal in need of such treatment, which comprises administering to said mammal a compound of formula I in an amount which is effective for treating said disease or condition.

When administered to a patient for the treatment of a disease in which a cytokine or cytokines are implicated, the dosage used can be varied within wide limits, depending upon the type of disease, the age and general condition of the patient, the particular compound administered, the presence or level of toxicity or adverse effects experienced with the drug and other factors. A representative example of a suitable dosage range is from as low as about 0.01 mg/kg to as high as about 100 mg/kg. However, the dosage administered is generally left to the discretion of the physician.

The methods of treatment can be carried out by delivering the compound of formula I parenterally. The term 'parenteral' as used herein includes intravenous, intramuscular, or intraperitoneal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred. The instant invention can also be carried out by delivering the compound of formula I subcutaneously, intranasally, intrarectally, transdermally or intravaginally.

The compounds of formula I may also be administered by inhalation. By 'inhalation' is meant intranasal and oral inhalation administration. Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by convention techniques.

The invention also relates to a pharmaceutical composition comprising a compound of formula I and a pharmaceutically acceptable carrier. The compounds of formula I may also be included in pharmaceutical compositions in combination with a second therapeutically active compound.

The pharmaceutical carrier employed may be, for example, either a solid, liquid or gas. Examples of solid carriers include lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Examples of liquid carriers are syrup, peanut oil, olive oil, water and the like. Examples of gaseous carriers include carbon dioxide and nitrogen.

Similarly, the carrier or diluent may include time delay material well known in the art, such as glyceryl monostearate or glyceryl distearate, alone or with a wax.

A wide variety of pharmaceutical dosage forms can be employed. If a solid dosage is used for oral administration, the preparation can be in the form of a tablet, hard gelatin capsule, troche or lozenge. The amount of solid carrier will vary widely, but generally will be from about 0.025 mg to about 1 g. When a liquid dosage form is desired for oral administration, the preparation is typically in the form of a syrup, emulsion, soft gelatin capsule, suspension or solution. When a parenteral dosage form is to be employed, the drug may be in solid or liquid form, and may be formulated for administration directly or may be suitable for reconstitution.

Topical dosage forms are also included. Examples of topical dosage forms are solids, liquids and semi-solids. Solids would include dusting powders, poultices and the like. Liquids include solutions, suspensions and emulsions. Semi-solids include creams, ointments, gels and the like.

The amount of a compound of formula I used topically will, of course, vary with the compound chosen, the nature and severity of the condition, and can be varied in accordance with the discretion of the physician. A representative, topical,

dose of a compound of formula I is from as low as about 0.01 mg to as high as about 2.0 g, administered one to four, preferably one to two times daily.

The active ingredient may comprise, for topical administration, from about 0.001% to about 10% w/w.

Drops according to the present invention may comprise sterile or non-sterile aqueous or oil solutions or suspensions, and may be prepared by dissolving the active ingredient in a suitable aqueous solution, optionally including a bactericidal and/or fungicidal agent and/or any other suitable preservative, and optionally including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 98-100°C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container aseptically. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

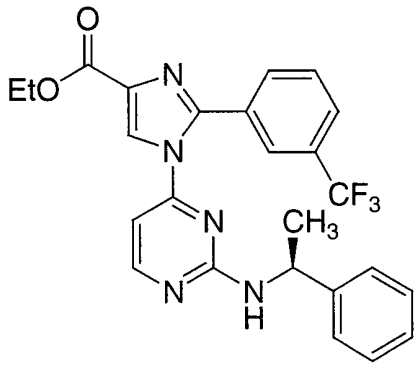
Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous liquid, with a greasy or non-greasy base. The base may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogels. The formulation may incorporate any suitable surface active agent such as

an anionic, cationic or non-ionic surfactant such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as silicas, and other ingredients such as lanolin may also be included.

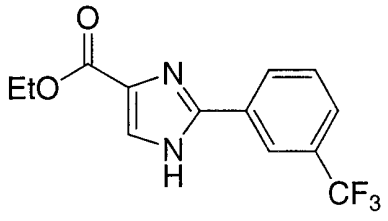
The following examples illustrate the preparation of some of the compounds of the invention and are not to be construed as limiting the invention disclosed herein.

EXAMPLE 1

(S)-1-[2-(1-Phenylethylamino)pyrimidin-4-yl]-2-(3-trifluoromethyl-phenyl)-1H-imidazole-4-carboxylic acid ethyl ester



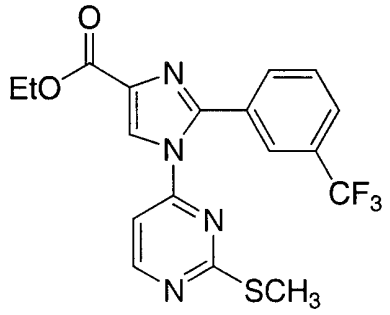
Step 1A: 2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid ethyl ester



A mixture of 3-trifluoromethylbenzamidoxime (19.89 g, 0.0974 mol) and ethyl propiolate (9.60 g, 0.0974 mol) in 100 mL methanol was refluxed overnight. The reaction was cooled and concentrated in vacuo and trace methanol removed by azeotroping with toluene (2 X 100 mL). The remaining residue was heated at 200°C for 6h in diphenyl ether (50 mL) before cooling and diluting with cold methanol (200 mL). The solid precipitate was filtered off and sucked dry on a glass frit to give 6.2 g of the title compound as a solid.

NMR (300MHz, CD_3OD) d: 8.32 (s, 1H), 8.20 (d, 1H), 7.92 (bs, 1H), 7.78-7.65 (m, 2H), 4.37 (q, 2H), 1.40 (t, 3H).

Step 1B: 1-(2-methylsulfanylpyrimidin-4-yl)-2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid ethyl ester

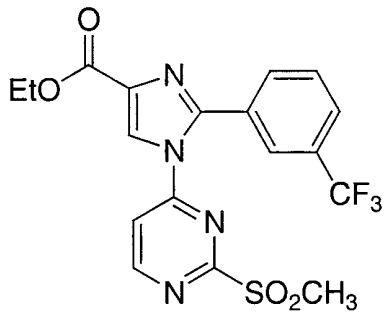


To a solution of 2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid ethyl ester (6.0 g, 0.0211 mol) in dimethyl formamide (60 mL) was added a 60% dispersion of sodium hydride in mineral oil (1.01 g, 0.0253 mol) and stirring was continued until noticeable H_2 evolution stopped. 4-Chloro-2-methylthiopyrimidine (3.73 g, 0.0232 mol) was added dropwise and the reaction heated to 120°C for 2h and 140°C overnight. The reaction was cooled and partitioned between H_2O (300 mL) and ethyl acetate (300 mL). A small (30 mL) portion of 10% citric acid solution was added before the layers were shaken and separated. The ethyl acetate layer was dried over anhydrous magnesium sulfate and then concentrated to an

oil which was chromatographed on silica using 15-35% ethyl acetate in hexanes to yield 6.43 g (75%) of the title compound as an oil.

NMR (300MHz, CD_3OD) d: 8.48 (d, 1H), 8.28 (s, 1H), 7.85 (s, 1H), 7.72-7.48 (m, 3H), 6.60 (d, 1H), 4.45 (q, 2H), 2.20 (s, 3H), 1.20 (t, 3H).

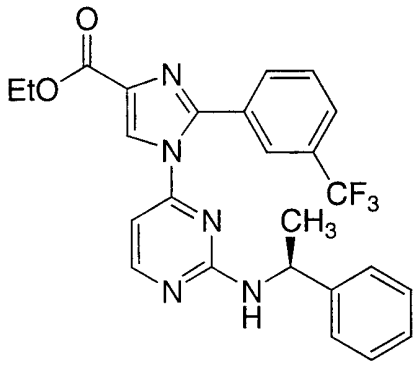
Step 1C: 1-(2-methylsulfonylpyrimidin-4-yl)-2-(3-trifluoro-methylphenyl)-1H-imidazole-4-carboxylic acid ethyl ester



A solution of Oxone® (25 g, 0.0406 mol) in water (100 mL) was added to a stirring solution of 1-(2-methyl sulfanylpyrimidin-4-yl)-2-(3-trifluoromethyl-phenyl)-1H-imidazole-4-carboxylic acid ethyl ester (6 g, 0.0146 mol) in 20 mL MeOH and 30 mL acetone. A mild exotherm was noted and the reaction stirred at room temperature for 2h before warming to 60°C for 4h. The resultant mixture was partitioned between ethyl acetate (600 mL) and sat. aq. $NaHCO_3$ solution (800 mL). The ethyl acetate was dried over anhydrous magnesium sulfate and concentrated to produce 5.3 g of the title compound as a solid used in the next step without further purification.

NMR (300MHz, CD_3OD) d: 9.05 (d, 1H), 8.62 (s, 1H), 7.92 (s, 1H), 7.86-7.62 (m, 4H), 4.40 (q, 2H), 3.05 (s, 3H), 1.40 (t, 3H).

Step 1D: (S)-1-[2-(1-phenylethylamino)pyrimidin-4-yl]-2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid ethyl ester

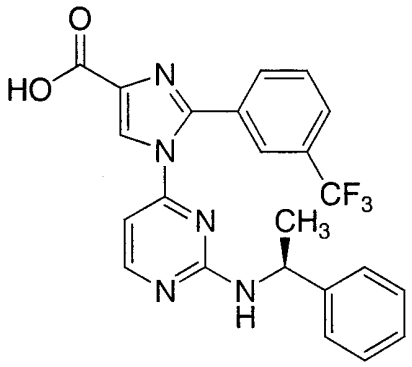


A solution of 1-(2-methylsulfonylpyrimidin-4-yl)-2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid ethyl ester (4.5 g, 0.0102 mol) in (S)-α-methylbenzylamine (20 mL) was stirred at room temperature for 30 min.. The reaction was partitioned between 300 mL ethyl acetate and 300 mL pH 4.5 citric acid solution (buffered with NaOH) and the ethyl acetate dried over anhydrous magnesium sulfate. Concentration of the ethyl acetate afforded 4.8 g of the title compound. A portion of this material was purified by reverse phase HPLC on C18 silica eluting with a gradient of 100% water to 30% water: 70% acetonitrile (both containing 0.1% trifluoroacetic acid.

NMR (300MHz, $CDCl_3$) d: 8.00 (bs, 1H), 7.82 (m, 2H), 7.66-7.20 (m, 9H), 6.12 (d, 1H), 4.72 (bs, 1H), 4.40 (q, 2H), 1.45 (bs, 3H), 1.40 (t, 3H).

EXAMPLE 2

(S)-1-[2-(1-phenylethylamino)pyrimidin-4-yl]-2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid



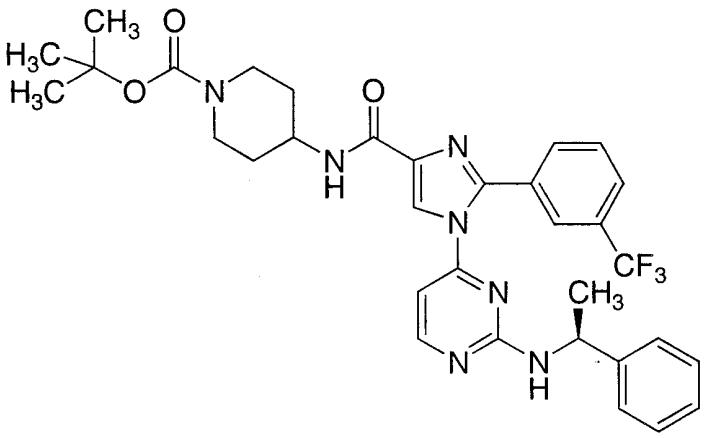
LiOH (3.75 g, 0.1566 mol) dissolved in a minimum amount of water was added to (S)-1-[2-(1-phenylethylamino)pyrimidin-4-yl]-2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid ethyl ester (4.2 g, 0.0087 mol) in 20 mL THF. 3 mL MeOH was added and the solution warmed to 60°C for 45 min.. After cooling to room temperature, the reaction was acidified with pH 4.5 citric acid solution (buffered with NaOH) and extracted with ethyl acetate (2 X 150 mL). The ethyl acetate was dried over anhydrous magnesium sulfate and concentrated to give 3.5 g of a solid. The solid was stirred vigorously for 30 minutes as a slurry in Et_2O and filtered to give the title compound (2.5 g) as a solid.

NMR (300MHz, CD_3OD) d: 8.36-8.24 (m, 2H), 7.88-7.60 (m, 4H), 7.30-7.14 (m, 5H), 6.58 (bs, 1H), 4.40 (bs, 1H), 1.40 (bs, 3H).

EXAMPLE 3

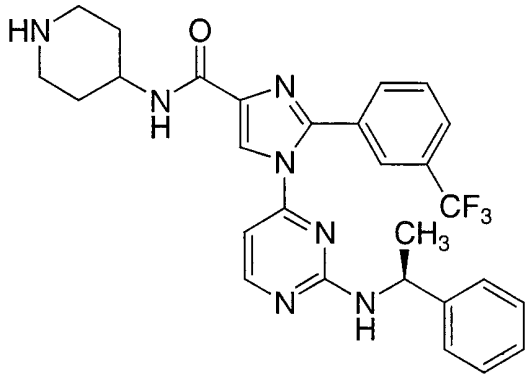
(S)-1-[2-(1-phenylethylamino)pyrimidin-4-yl]-2-(3-trifluoromethyl-phenyl)-1H-imidazole-4-carboxylic acid piperidin-4-ylamide

Step 3A: (S)-1-[2-(1-phenylethylamino)-pyrimidin-4-yl]-2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid -N-t-butoxycarbonylpiperidin-4-ylamide



Benzotriazol-1-yloxy-tris-(dimethylamino)-phosphonium-hexafluoro phosphate (0.585 g, 1.32 mmol) was added to 3 mL dimethyl formamide containing (S)-1-[2-(1-phenylethylamino)-pyrimidin-4-yl]-2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid (0.500 g, 1.10 mmol) and the solution stirred during the addition of N-methylmorpholine (0.560 g, 5.51 mmol). N-*tert*-butoxycarbonyl-4-aminopiperidine (0.270 g, 1.34 mmol) in 3 mL dimethylformamide was added and the reaction stirred for 16 h at room temperature. The mixture was partitioned between ethyl acetate (50 mL) and H_2O (50 mL). The ethyl acetate layer was then washed with 10% citric acid solution (50 mL), 10% $NaHCO_3$ (50 mL) and 2 X 40 mL H_2O before being dried over anhydrous magnesium sulfate. Concentration of the ethyl acetate yielded 630 mg ofthe title compound as a solid.

Step 3B: (S)-1-[2-(1-phenylethylamino)pyrimidin-4-yl]-2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid piperidin-4-ylamide

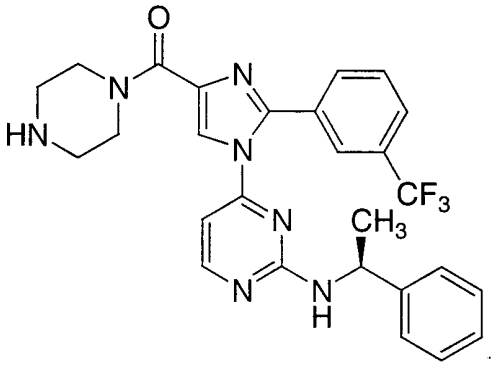


(S)-1-[2-(1-Phenyl-ethylamino)-pyrimidin-4-yl]-2-(3-trifluoromethyl-phenyl)-1H-imidazole-4-carboxylic acid -N-t-butoxycarbonylpiperidin-4-ylamide (600mg) was dissolved in 10 mL CH_2Cl_2 and cooled to 0°C before adding 3 mL TFA. The reaction was allowed to warm to room temperature and stirred for 1h. Concentration and prep HPLC on a Delta-Pak™ 40mm X 200mm C_{18} column from 100/0 H_2O/CH_3CN to 5/95 H_2O/CH_3CN over30 min yielded 280 mg of the title compound after lyophilization as a granular solid.

NMR (300MHz, CD_3OD) d: 8.30 (d, 1H, J=5Hz), 8.12 (s, 1H), 7.87 (s, 1H), 7.76 (d, 1H, J=7.5Hz), 7.65-7.55 (m, 2H), 7.27-7.16 (m, 5H), 6.49 (bs, 1H), 4.41 (bs, 1H), 4.18 (m, 1H), 3.53-3.44 (m ,2H), 3.20-3.11 (m, 2H), 2.22-2.17 (m ,2H), 1.95-1.81 (m, 2H), 1.36 (s, 3H)

EXAMPLE 4

{(S)-1-[2-(1-Phenylethylamino)pyrimidin-4-yl]-2-(3-trifluoromethylphenyl)-1H-imidazol-4-yl}-piperazine-1-yl-amide

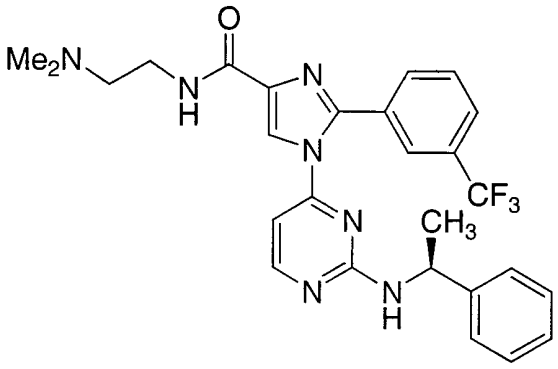


The title compound was prepared using the procedures described in example 3, replacing N-*tert*-butoxycarbonyl-4-aminopiperidine with N-*tert*-butoxycarbonyl piperazine.

NMR (300MHz, CD_3OD) d: 8.32 (bs, 1H, 8.12 (bs, 1H), 7.79-7.74 (m, 2H), 7.67-7.56 (m, 2H), 7.24-7.16 (m, 5H), 6.49 (s, 1H), 4.86-3.53 (m, 8H), 1.35 (s, 3H)

EXAMPLE 5

(S)-1-[2-(1-phenylethylamino)pyrimidin-4-yl]-2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid (2-dimethylaminoethyl)amide

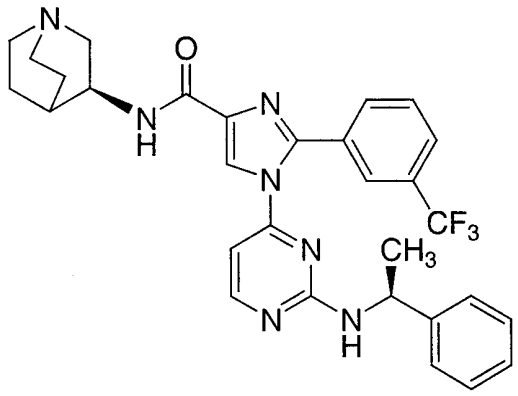


The title compound was prepared using the procedure described in example 3 step A, replacing N-*tert*-butoxycarbonyl-4-aminopiperidine with N,N-dimethylethylenediamine

NMR (300MHz, CD_3OD) d: 8.31 (bs, 1H), 8.13 (s, 1H), 7.87 (s, 1H), 7.75 (d, 1H, J=7.3Hz), 7.64-7.54 (m, 2H), 7.23-7.16 (m, 5H), 6.52 (s, 1H), 4.40 (bs, 1H), 3.77 (t, 2H), 3.40 (t, 2H), 2.98 (s, 6H), 1.35 (bs, 3H)

EXAMPLE 6

(S)-1-[2-(1-phenylethylamino)pyrimidin-4-yl]-2-(3-trifluoromethyl-phenyl)-1H-imidazole-4-carboxylic acid (1-azabicyclo[2.2.2]oct-3R-yl)-amide

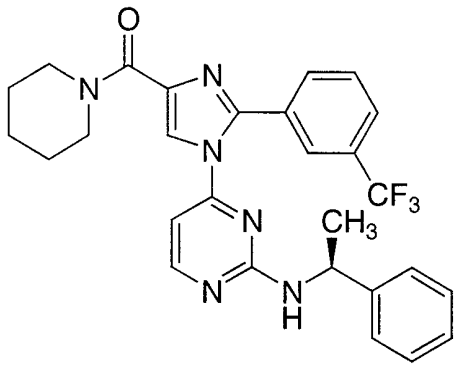


The title compound was prepared using the procedure described in example 3 step A, replacing N-*tert*-butoxycarbonyl-4-aminopiperidine with (R)-3-amino bicyclo[2.2.2]octane.

NMR (300MHz, CD_3OD) d: 8.31 (bs, 1H), 8.15 (s, 1H), 7.89 (s, 1H), 7.77 (d, 1H, J=7Hz), 7.65-7.56 (m, 2H), 7.24-7.16 (m, 5H), 6.51 (s, 1H), 4.48 (m, 2H), 3.77 (m, 1H), 3.53-3.29 (m, 4H), 2.37-2.27 (m, 2H), 2.12-1.94 (m, 3H), 1.35 (s, 3H)

EXAMPLE 7

1-[2-(1-phenylethylamino)pyrimidin-4-yl]-2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid piperidine amide

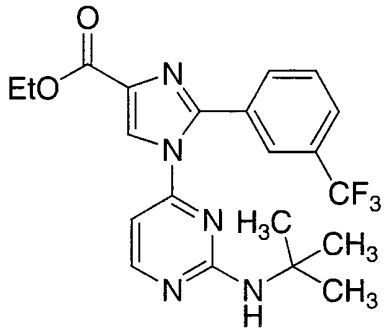


The title compound was prepared using the procedure described in example 3 step A, replacing N-*tert*-butoxycarbonyl-4-aminopiperidine with piperidine.

m/z 521.2

EXAMPLE 8

1-[2-(t-butylamino)pyrimidin-4-yl]-2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid ethyl ester

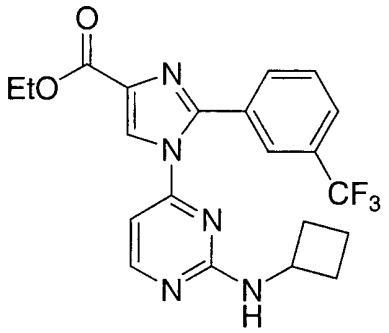


The title compound was prepared as described in Example 1 step D, replacing (S)-α-methylbenzylamine with t-butylamine.

NMR (300MHz, CD_3OD) d: 8.36 (d, 1H, J=5.4Hz), 8.31 (s, 1H), 7.81-7.60 (m, 4H), 6.58 (s, 1H), 4.40 (q, 2H), 1.40 (t, 3H), 1.18 (s, 9H)

EXAMPLE 9

1-[2-(cyclobutylamino)pyrimidin-4-yl]-2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid ethyl ester

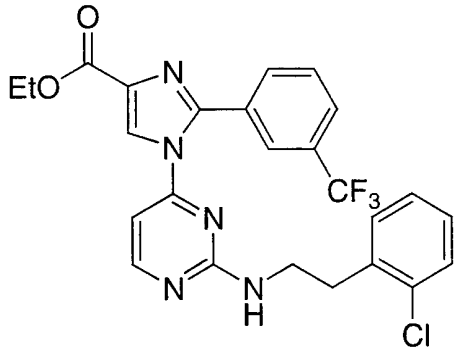


The title compound was prepared as described in Example 1 step D, replacing (S)-α-methylbenzylamine with cyclobutylamine.

NMR (300MHz, CD_3OD) d: 8.39 (s, 1H), 8.33 (d, 1H, J=5.3Hz), 7.83-7.63 (m, 4H), 6.69 (s, 1H), 4.40 (q, 2H), 3.60 (bs, 1H), 2.20-1.40 (m, 6H), 1.37 (t, 3H)

EXAMPLE 10

1-[2-(2-(2-chlorophenyl)ethylamino)pyrimidin-4-yl]-2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid ethyl ester

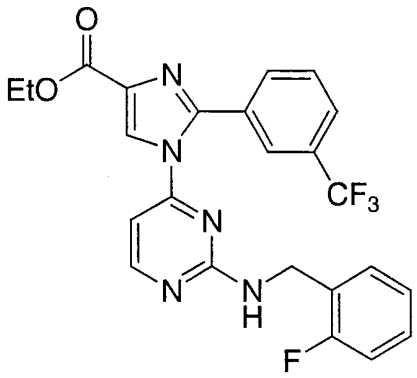


The title compound was prepared as described in Example 1 step D, replacing (S)-α-methylbenzylamine with 2-(2-chlorophenyl) ethylamine.

NMR (300MHz, CD_3OD) d: 8.22 (m, 2H), 7.85 (s, 1H), 7.65 (m, 2H), 7.52-7.31 (m, 2H), 7.18 (m, 4H), 6.17 (m, 1H), 5.39 (bs, 1H), 4.45 (q, 2H), 3.80-3.41 (m, 2H), 3.10-2.85 (m, 2H), 1.43 (t, 3H)

EXAMPLE 11

1-[2-((2-fluorobenzyl)amino)pyrimidin-4-yl]-2-(3-trifluoromethylphenyl)-1H-imidazole-4-carboxylic acid ethyl ester

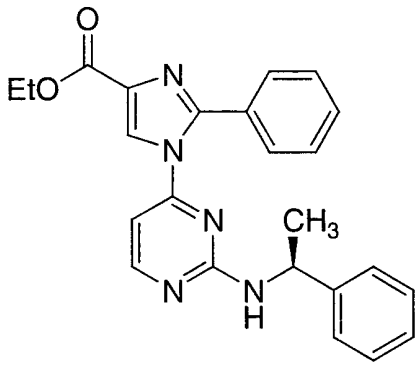


The title compound was prepared as described in Example 1 step D, replacing (S)-α-methylbenzylamine with 2-fluorobenzylamine.

NMR (300MHz, CD_3OD) d: 8.35-8.29 (m, 2H), 7.80-7.56 (m, 4H), 7.34-7.00 (m, 4H), 6.57 (bs, 1H), 4.40 (q, 2H), 4.20 (bs, 2H), 1.40 (t, 3H)

EXAMPLE 12

(S)-1-[2-(1-Phenylethylamino)pyrimidin-4-yl]-2-phenyl-1H-imidazole-4-carboxylic acid ethyl ester

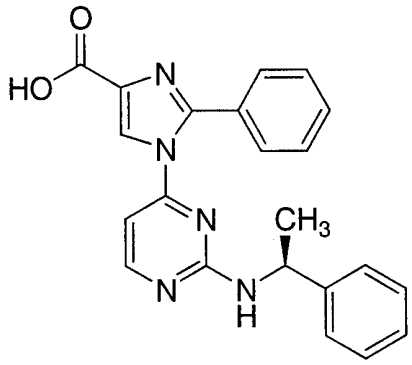


The title compound was prepared as described in Example 1 steps A to D, replacing 3-trifluoromethylbenzamidoxime in step A with benzamidoxime

NMR (300MHz, CD_3OD) d: 8.21 (d, 1H, J=4.9Hz), 8.11 (bs, 1H), 7.54-7.42 (m, 5H), 7.34-7.26 (m, 4H), 7.24-7.16 (m, 1H), 6.28 (bs, 1H), 4.65 (bs, 1H), 4.40 (q, 2H, J=7.1Hz), 1.43 (m, 6H)

EXAMPLE 13

(S)-1-[2-(1-Phenylethylamino)pyrimidin-4-yl]-2-phenyl-1H-imidazole-4-carboxylic acid



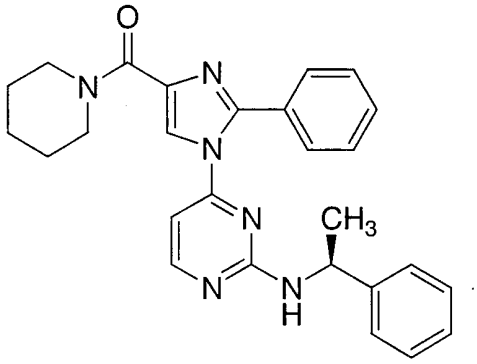
Utilizing the procedure described in example 2, the title compound was prepared from example 13.

NMR (300MHz, CD_3OD) d: 8.22 (d, 1H, J=5.1Hz), 8.18 (bs, 1H), 7.58-7.42 (m, 5H), 7.32-7.24 (m, 4H), 7.23-7.14 (m, 1H), 6.31 (bs, 1H), 4.63 (bs, 1H), 1.18 (d, 3H, J=7.1Hz)

EXAMPLE 14

(S)-1-[2-(1-Phenylethylamino)pyrimidin-4-yl]-2-

phenyl-1H-imidazole-4-carboxylic acid piperidine amide

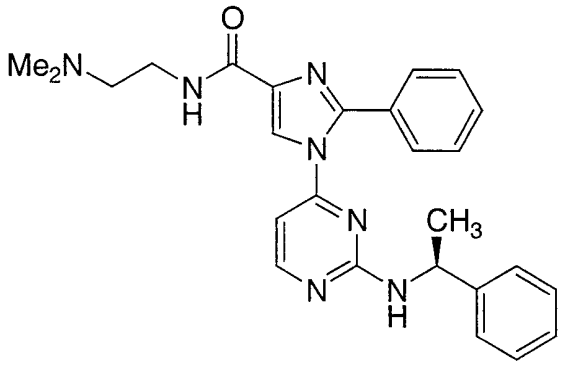


Utilizing the procedure described in example 7, the title compound was prepared from example 13.

NMR (300MHz, CD_3OD) d: 8.18 (d, 1H, 4.9Hz), 7.96 (bs, 1H), 7.43 (m, 5H), 7.27-7.18 (m, 5H), 6.25 (bs, 1H), 4.75 (bs, 1H), 3.96 (m, 2H), 3.71 (m, 2H), 1.74-1.65 (m, 6H), 1.43 (m, 3H)

EXAMPLE 15

(S)-1-[2-(1-Phenylethylamino)pyrimidin-4-yl]-2-phenyl-1H-imidazole-4-carboxylic acid (2-dimethylaminoethyl) amide

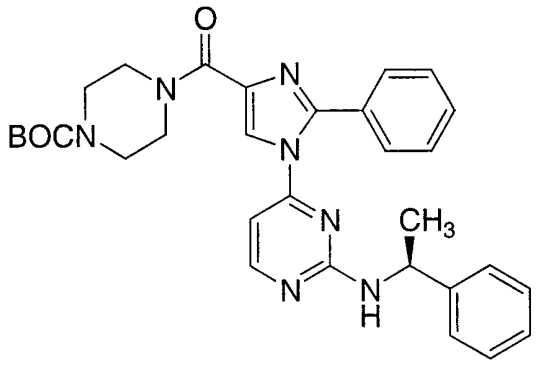


Utilizing the procedure described in example 5, the title compound was prepared from example 13.

NMR (300MHz, CD_3OD) d: 8.22 9d, 1H, 5.13 Hz), 8.12 (bs, 1H), 7.48-7.44 (m, 5H), 7.40-7.15 (m, 5H), 6.29 (s, 1H), 4.88 (bs, 1H), 3.79 (t, 2H, 5.7Hz), 3.38 (t, 2H, 5.7Hz), 3.06 (s, 6H), 1.43 (s, 3H)

EXAMPLE 16

{(S)-1-[2-(1-Phenylethylamino)-pyrimidin-4-yl]-2-phenyl-1H-imidazol-4-yl}-4-(benzyloxycarbonylpiperazine)-1-yl-amide



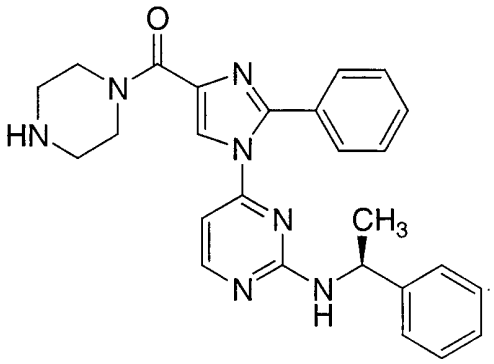
Utilizing the procedure described in example 3 step A, the title compound was prepared from example 13.

NMR (300MHz, CD_3OD) d: 8.22 (bs, 1H), 8.04 (bs, 1H), 7.51-7.43 (m, 5H), 7.27-7.19 (m, 5H), 6.37 (bs, 1H), 4.62 (bs, 1H), 4.06-3.77 (m, 8H), 1.48-1.35 (m, 12H)

EXAMPLE 17

{(S)-1-[2-(1-Phenylethylamino)-pyrimidin-4-yl]-2-

phenyl-1H-imidazol-4-yl}-4-(piperazine)-1-yl-amide



Utilizing the procedure described in example 3 step B, the title compound was prepared from example 16.

NMR (300MHz, CD_3OD) d: 8.22 (s, 1H), 8.07 (bs, 1H), 7.48-7.42 (m, 5H), 7.28-7.17 (m, 5H), 6.29 (s, 1H), 4.87-3.80 (m, 8H), 1.28 (s, 3H)

The ability of compounds of the present invention to inhibit the synthesis or the activity of cytokines can be demonstrated using the following *in vitro* assays.

BIOLOGICAL ASSAYS

Lipopolysaccharide mediated production of cytokines

Human peripheral blood mononuclear cells (PBMC) are isolated from fresh human blood according to the procedure of Chin and Kostura, *J. Immunol.* **151,** 5574-5585 (1993). Whole blood is collected by sterile venipuncture into 60 mL syringes coated with 1.0 mL of sodium- heparin (Upjohn, 1000 U/mL) and diluted 1:1 in Hanks Balanced Salt Solution (Gibco). The erythrocytes are separated from the PBMC's by centrifugation on a Ficoll-Hypaque lymphocyte separation media. The PBMC's are washed three times in Hanks Balanced Salt Solution and then

resuspended to a final concentration of 2 x 10^6 cell/mL in RPMI containing 10% fresh autologous human serum, penicillin streptomycin (10 U/mL) and 0.05% DMSO. Lipopolysaccharide (Salmonella type Re545; Sigma Chemicals) is added to the cells to a final concentration of 100 ng/mL. An aliquot (0.1 mL) of the cells is quickly dispensed into each well of a 96 well plate containing 0.1 mL of the test compound, at the appropriate dilution, and are incubated for 24 hours. at 37°C in 5% CO2 . At the end of the culture period, cell culture supernatants are assayed for IL-1β , TNF-α, IL-6 and PGE2 production using specific ELISA.

IL-1 mediated cytokine production

Human peripheral blood mononuclear cells are isolated from fresh human blood according to the procedure of Chin and Kostura, *J. Immunol.* **151,** 5574-5585 (1993). Whole blood is collected by sterile venipuncture into 60 mL syringes coated with 1.0 mL of sodium- heparin (Upjohn, 1000 U/mL) and diluted 1:1 in Hanks Balanced Salt Solution (Gibco). The erythrocytes are separated from the PBMC's by centrifugation on a Ficoll-Hypaque lymphocyte separation media. The PBMC's are washed three times in Hanks Balanced Salt Solution and then resuspended to a final concentration of 2 x 10^6 cell/mL in RPMI containing 10% fresh autologous human serum, penicillin streptomycin (10 U/mL) and 0.05% DMSO. Endotoxin free recombinant human IL-1b is then added to a final concentration of 50 pMolar. An aliquot (0.1 mL) of the cells is quickly dispensed into each well of a 96 well plate containing 0.1 mL of the compound at the appropriate dilution. and are incubated for 24 hours. at 37°C in 5% CO2 . At the end of the culture period, cell culture supernatants are assayed for TNF-a, IL-6 and PGE2 synthesis using specific ELISA.

Determination of IL-1β , TNF-α, IL-6 and prostanoid production from LPS or IL-1 stimulated PBMC's

IL-1β ELISA

Human IL-1β can be detected in cell-culture supernatants or whole blood with the following specific trapping ELISA. Ninety-six well plastic plates (Immulon 4; Dynatech) are coated for 12 hours at 4°C with 1 mg/mL protein-A affinity chromatography purified mouse anti-human IL-1β monoclonal antibody (purchased as an ascites preparation from LAO Enterprise, Gaithersburg Maryland.) diluted in Dulbecco's phosphate-buffered saline (-$MgCl_2$, -$CaCl_2$). The plates are washed with PBS-Tween (Kirkegaard and Perry) then blocked with 1% BSA diluent and blocking solution (Kirkegaard and Perry) for 60 minutes at room temperature followed by washing with PBS Tween. IL-1β standards are prepared from purified recombinant IL-1β produced from E. *coli*.. The highest concentration begins at 10 ng/mL followed by 11 two-fold serial dilutions. For detection of IL-1β from cell culture supernatants or blood plasma, 10 - 25 mL of supernatant is added to each test well with 75-90 mL of PBS Tween. Samples are incubated at room temperature for 2 hours then washed 6 times with PBS Tween on an automated plate washer (Dennly). Rabbit anti-human IL-1β polyclonal antisera diluted 1:500 in PBS-Tween is added to the plate and incubated for 1 hour at room temperature followed by six washes with PBS-Tween. Detection of bound rabbit anti-IL-1β IgG is accomplished with Fab' fragments of Goat anti-rabbit IgG-horseradish peroxidase conjugate (Accurate Scientific) diluted 1:10,000 in PBS-Tween. Peroxidase activity was determined using TMB peroxidase substrate kit (Kirkegaard and Perry) with quantitation of color intensity on a 96-well plate Molecular Devices spectrophotometer set to determine absorbance at 450 nM. Samples are evaluated using a standard curve of absorbance versus concentration. Four-parameter logistics analysis generally is used to fit data and obtain concentrations of unknown compounds.

TNF-α ELISA

Immulon 4 (Dynatech) 96-well plastic plates are coated with a 0.5 mg/mL solution of mouse anti-human TNF-a monoclonal antibody. The secondary antibody is a 1:2500 dilution of a rabbit anti-human TNF-α polyclonal serum purchased from Genzyme. All other operations are identical to those described above

for IL-1β. The standards are prepared in PBS-Tween + 10% FBS or HS. Eleven 2 fold dilutions are made beginning at 20 ng/mL TNF-α.

IL-6 ELISA

Levels of secreted human IL-6 are also determined by specific trapping ELISA as described previously in Chin and Kostura, *J. Immunol.* **151,** 5574-5585 (1993). (Dynatech) ELISA plates are coated with mouse anti-human IL-6 monoclonal antibody diluted to 0.5 mg/mL in PBS. The secondary antibody, a rabbit anti-human IL-6 polyclonal antiserum, is diluted 1:5000 with PBS-Tween. All other operations are identical to those described above for IL-1b. The standards are prepared in PBS-Tween + 10% FBS or HS. Eleven 2 fold dilutions are made beginning at 50 ng/mL IL-6.

PGE_2 production

Prostaglandin E2 is detected in cell culture supernatants from LPS or IL-1 stimulated PBMC's using a commercially available enzyme immunoassay . The assay purchased from the Cayman Chemical (Catalogue number 514010) and is run exactly according to the manufacturers instructions.

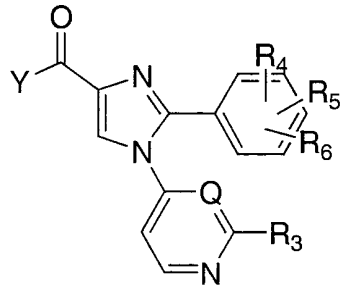
Interleukin8 (IL-8)

The present compounds can also be assayed for IL-8 inhibitory activity as discussed below. Primary human umbilical cord endothelial cells (HUVEC) (Cell Systems, Kirland, Wa) are maintained in culture medium supplemented with 15% fetal bovine serum and 1% CS-HBGF consisting of aFGF and heparin. The cells are then diluted 20-fold before being plated (250 μl) into gelatin coated 96-well plates. Prior to use, culture medium is replaced with fresh medium (200μl). Buffer or test compound (25μl, at appropriate concentrations) is then added to each well in quadruplicate wells and the plates incubated for 6h in a humidified incubator at 37°C in an atmosphere of 5% CO_2. At the end of the incubation period, supernatant is removed and assayed for IL-8 concentration using an IL-8 ELISA kit obtained from R&D Systems (Minneapolis, MN). All data is

presented as mean value (ng/mL) of multiple samples based on the standard curve. IC50 values where appropriate are generated by non-linear regression analysis.

WHAT IS CLAIMED IS:

1. A compound of the formula



(I)

wherein

Y is OH, O(C_1- C_6 alkyl), NR_1R_2, N(C_1- C_6 alkyl), each alkyl being optionally substituted by 1-3 groups selected from halogen, hydroxy, CF_3, NH_2, and NO_2; or a heterocyclic group connected to the carbonyl group by a direct bond, NH or by C_1-C_6 alkyl, with the proviso that both R_1 and R_2 cannot be hydrogen;

R_1 and R_2 are independently hydrogen, C_1-C_6 alkyl, (C_1- C_6 alkyl)-N-(C_1-C_6 alkyl)$_p$; said alkyl being optionally substituted by 1-3 groups selected from halogen, hydroxy, CF_3, NH_2, and NO_2; or

R_1 and R_2 are taken together to form an optionally substituted 4 to 10 membered mono or bicyclic heterocycle ring containing at least one N atom, and optionally containing 1-2 additional N atoms and 0-1 O or S atoms, said ring optionally substituted by 1-3 groups selected from C_1- C_4alkyl, OH, O(C_1- C_6 alkyl),

R_3 is hydrogen, NH(C_1-C_6 alkyl)aryl, NH(C_1-C_6 alkyl) or NH(C_3-C_6 cycloalkyl), said aryl group being optionally substituted by 1-3 groups selected from halogen, hydroxy, CF_3, NH_2, and NO_2;

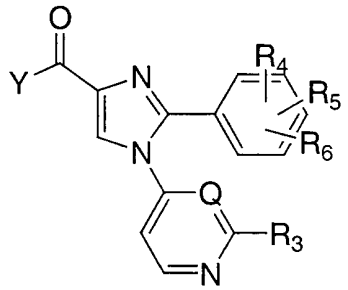
R_4, R_5 and R_6 independently represent a member selected from the group consisting of hydrogen, halogen, hydroxy, CF_3, NH_2, NO_2, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl or aryl;

Q is CH or N;

p is 0-2;

or a pharmaceutically acceptable addition salt and/or hydrate thereof, or where applicable, a geometric or optical isomer or racemic mixture thereof.

2. The compound in accordance with claim 1 of the formula



(I)

or a pharmaceutically acceptable salt, or optical isomer thereof,

wherein:

Y is OH, O(C_1- C_6 alkyl), NR_1R_2 or a piperidinyl or piperazinyl group connected to the imidazole ring by a direct bond;

R_1 and R_2 are independently hydrogen or $(CH_3)_2$ - N- $(CH_3)_2$; or

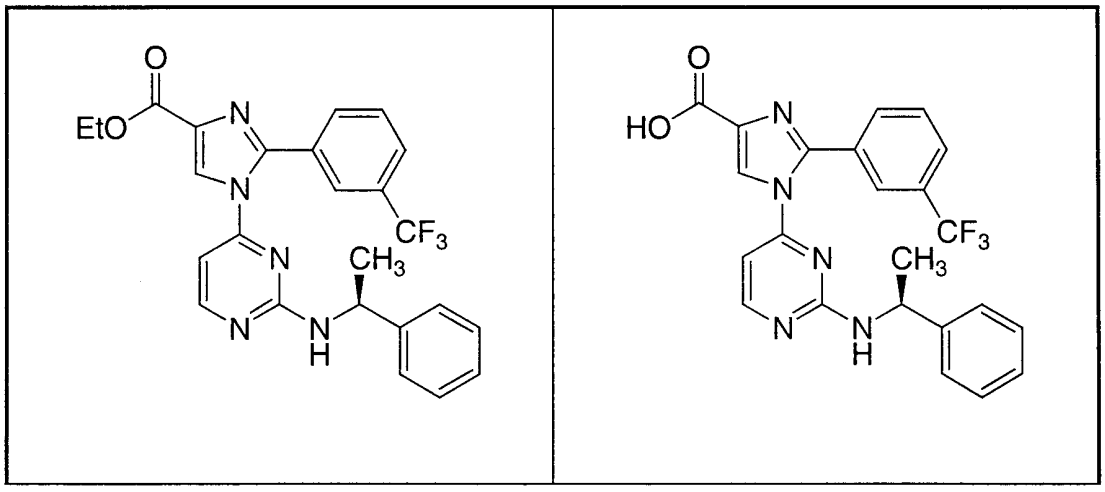
R_1 and R_2 are taken together to form a heterobicylic ring;

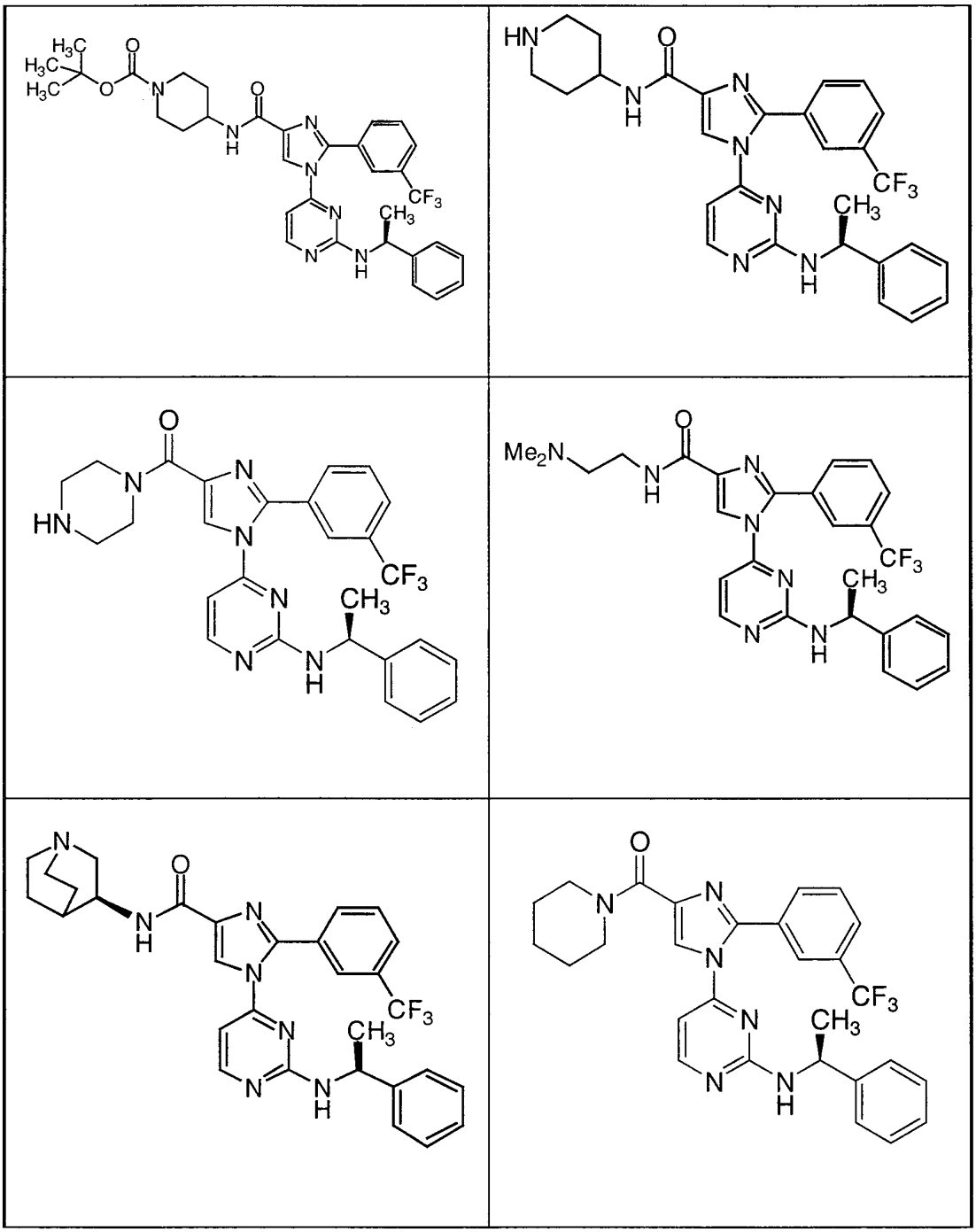
R_4 is CF_3

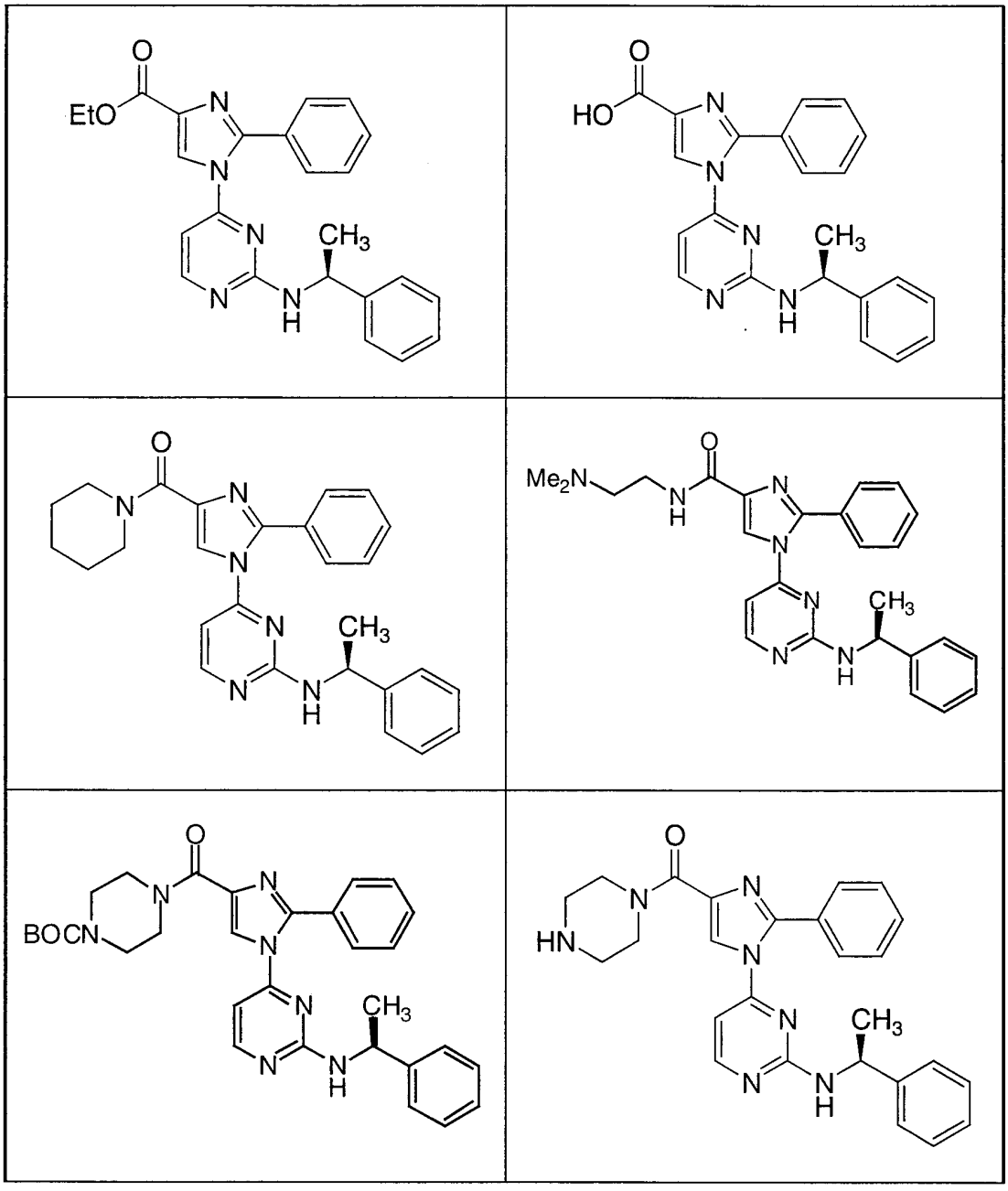
R_5 and R_6 are hydrogen; and

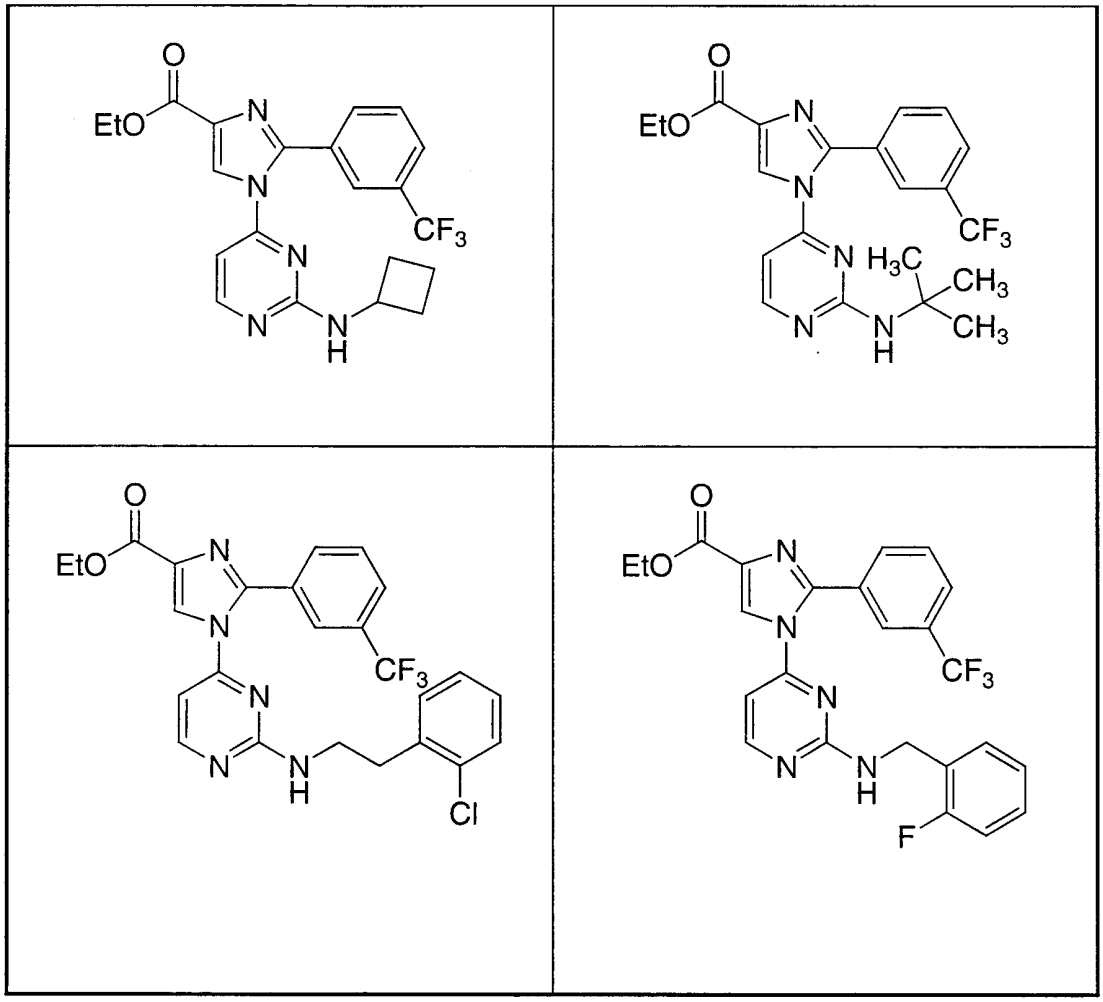
Q is N.

3. A compound of the formula









or a pharmaceutically acceptable addition salt and/or hydrate thereof, or where applicable, a geometric or optical isomer or racemic mixture thereof.

4. A pharmaceutical composition which is comprised of a compound in accordance with claim 1 in combination with a pharmaceutically acceptable carrier.

5. A pharmaceutical composition which is produced

by combining a compound in accordance with claim 1 and a pharmaceutically acceptable carrier.

6. A method of treating a cytokine mediated disease in a mammal, comprising administering to a mammalian patient in need of such treatment an amount of a compound as described in claim 1 in an amount which is effective to treat said cytokine mediated disease.

7. A method of treating inflammation in a mammalian patient in need of such treatment, which is comprised of administering to said patient an anti-inflammatory effective amount of a compound as described in claim 1.

8. A method in accordance with claim 6 wherein the cytokine mediated disease is rheumatoid arthritis, osteoarthritis, endotoxemia, toxic shock syndrome, inflammatory bowel disease, tuberculosis, atherosclerosis, muscle degeneration, cachexia, psoriatic arthritis, Reiter's syndrome, rheumatoid arthritis, gout, traumatic arthritis, rubella arthritis or acute synovitis.

9. A method in accordance with claim 6 wherein the cytokine mediated disease is rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcosis, bone resorption diseases, reperfusion injury, graft v. host rejection, allograft rejection, fever, myalgia due to infection, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), AIDS related complex (ARC), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis or pyresis.

10. A method of treating osteoporosis in a mammalian patient in need of such treatment, which is comprised of administering to said patient an amount of a compound as described in claim 1 which is effective to treat osteoporosis.

11. A method of treating bone resorption in a mammalian patient in need of such treatment, which is comprised of administering to said patient an amount of a compound as described in claim 1 which is effective to treat bone resorption.

12. A method of treating Crohn's disease in a mammalian patient in need of such treatment which is comprised of administering to said patient an amount of a compound as described in claim 1 which is effective to treat Crohn's disease.

13. A process for making a pharmaceutical composition comprising combining a compound of Claim 1 and a pharmaceutically acceptable carrier.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/12973

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :C07D 401/04, 403/04; A61K 31/4439, 31/506
US CL : 544/330, 331, 333; 546/274.1; 514/275, 256, 341
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 544/330, 331, 333; 546/274.1; 514/275, 256, 341

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN EXPRESS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,716,972 A (ADAMS et al. 10 February 1998 , col.3, lines 35,-36, 46-49, 54; col. 4, lines 1, 3, 19, 33-34.	1-13
A,P	US 5,955,480 A (CHANG) 21 September 1999, col.4, lines 50-67; col. 5, lines 7-11.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 24 JULY 2000	Date of mailing of the international search report 28 SEP 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JANE FAN Telephone No. (703) 308-2351

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/12973

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/12973

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1, 4-13 (all in part) wherein Q is CH, drawn to pyridyl derivatives, composition containing thereof and method of using.
Group II, claim(s) 2-3 and claims 1, 4-13 (all in part) wherein Q is N, drawn to pyrimidines, composition containing thereof and method of using.
The inventions listed as Groups I-II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the compounds have different core, furthermore pyridines have other utility as taught in pat' 5,716,972 by Adams et al., col. 3, line 22 treating HIV.